Chapter 5
Organ Culture of Embryonic Chick Heart

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This laboratory was originally designed by Dr. Jeanne A. Powell, Smith College, Northampton, Massachusetts, and an earlier version was published by Powell and Pitkin in *The American Biology Teacher,* Vol. 43, Number 1: January 1981.

Introduction

This laboratory exercise was designed to acquaint students with the embryonic development of the chick, fine dissection, organ culture, and application of experimental results to questions concerning development. It is intended for students in introductory biology and the developmental biology and embryology courses. The students first observe whole mounts of chick embryos to identify structures, paying special attention to the heart structures. Next they obtain a 55–72 hour chick embryo, dissect out the heart, and place it in culture medium. The second heart removed is dissected into three pieces and placed in culture medium. Heart rate is observed in both the whole explanted heart and in heart pieces before and after 18 hours of incubation in the culture medium. After incubation the students look for evidence of morphogenesis. See Figure 5.1 for an outline of the procedure.

Students in an introductory-level biology course should be able to complete this exercise easily in two to three hours. One observation 18 hours later is necessary and takes less than 15 minutes. Large classes can accomplish this exercise if sufficient equipment is available.

Initially the laboratory set up may take 4–5 hours if materials such as glass dissection equipment, fine forceps and scissors, and culture dishes are not part of the equipment routinely available to introductory laboratories. The single most important factor necessary to make this laboratory a successful experience is a *source of fertile eggs*. Fertile eggs may be obtained from the major biological supply houses, but they are very expensive. Finding fertile eggs in the local community may be time-consuming but will save a considerable amount of money.

Student Materials

Objectives

1. To become familiar with the structures visible at different stages of chick development.
2. To determine the heart structures, direction of blood flow, and heart rate of a 55-hour chick embryo.
3. To use sterile techniques and organ culture.
4. To answer the developmental questions posed in the exercise.

Introduction

In the chick, early development (cleavage) occurs before the egg is laid, but for matters of convenience the age of the embryo is usually described as so many hours of incubation at 37.5°C. The heart of the chick embryo de-

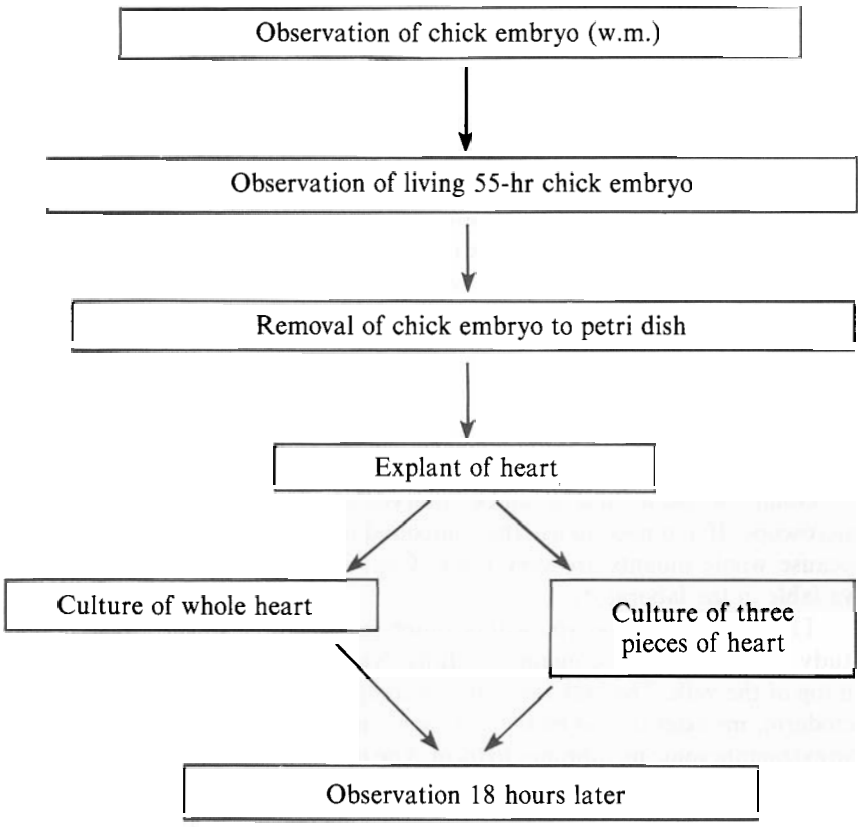

Figure 5.1. Flow diagram outlining procedures.

velops from the fusion of paired precardiac mesodermal tubes between 24 and 29 hours of incubation. The cells that compose these tubes have migrated over long distances to the heart-forming area. As the single heart tube fuses, and before the actual circulation of blood commences, the fused ventricular portion begins to pulsate (at about 29 hours of incubation). As fusion continues the atrial portion of the heart is incorporated, and the rate of the heart beat increases because of the controlling cells of the atrium (33 hours). Further fusion of the tubes continues as the heart becomes twisted in an S shape, and by 44 hours the sinus venosus is incorporated, and again the heart-beat rate changes. Following completion of fusion further changes in shape (morphogenesis) occur, transforming the simple S-shaped tube into a four-chambered heart.

Development of cells, tissues or organs in the living organism is called *in vivo* development, while that in culture is called *in vitro* development. In an

in vitro situation one can study the influence of chemicals and other living tissue on one particular tissue or organ when it is isolated from the influence of the body as a whole.

In explanting organ (heart) primordia of the chick embryo we are using essentially the method of organ culture. It has taken many years of experimentation with growth requirements to establish the type of medium that will supply a growing portion of an organism with all the requirements so that it can grow independently of the whole organism. The composition of the culture medium is listed under supplies. However, the full ingredients of the salt solution and basal medium are too complex to be included.

Exercise

A. Whole mounts of 24-hour, 33-hour, 48-hour, and 72-hour chick embryos.

Obtain whole mounts of chick embryos and observe using a dissecting microscope. If you need to use the compound microscope use only low power, because whole mounts are very thick! Compare your slides with drawings available in the laboratory.

The living chick that you will be observing is at about the 55-hour stage. Study the 48-hour whole mount carefully. Notice that the embryo is growing on top of the yolk. The yolk sac which is composed of all three germ layers—ectoderm, mesoderm, and endoderm (now only a small disc)—is the first of the extraembryonic membranes to form. The developing embryo is now turned on its side. It is made of a tubule of endoderm (gut), surrounded by mesoderm, which is surrounded by ectoderm. The nervous system has already begun to develop from folding of the neural plate. It is composed basically of a long tube with the brain craniad and the spinal cord caudad. The eye cup is an outgrowth of the brain, while the lens has formed from an ectodermal placode by invagination. The otocyst or future ear (inner) has also formed from the ectoderm.

The developing heart (formed from mesoderm) is prominent. It has developed from a tube and now takes on an S-shape configuration. Blood flows (in life) from the yolk sac to the sinus venosus, atrium, ventricle and aortic arches to the whole body and out to the yolk again, thereby transporting food and oxygen and disposing of waste material. Blood flow to and from the yolk sac can be observed in the artery and vein of the yolk. The amnion, chorion and allantois have not yet formed. Notice the developing somites (from which muscles and vertebrae will develop) on either side of the spinal cord. Identify the main features of the 48-hour chick so that you will be familiar with them in the living specimen.

B. Observation of the living 55-hour chick embryo.

Before you obtain your chick embryo, check all your equipment to be sure you have everything in readiness. Each pair of students will be provided with two eggs. The first, for gross observation and, if possible, first heart explantation. The second will be used for heart explantation.

Each pair of students should have a finger bowl, sterile petri dish, 2 fine forceps, fine scissors, and 3 sharp glass rods in Coplin jar with alcohol. Rinse your instruments in the alcohol to prevent contamination of your chick embryo. Do not touch the embryo with alcohol.

Obtain a 55-hour egg. Hold it in exactly the same orientation as it had in the tray. Pour about 1 cm of warm Howard's Ringer (HR) solution, a physiological saline solution, into a fingerbowl. Crack the egg on the side of the bowl. Carefully lower the egg into the Ringer's solution. THE OBJECT IS TO NOT BREAK THE YOLK. (If the yolk breaks at this point, try to remove the vasculated area—the area with blood vessels—to the large petri dish filled with warm HR.) If you did not break the yolk you should be able to see the heart beating using the dissecting microscope. On your chick embryo:

1. Identify the sinus venosus, atrium, and ventricle;
2. Determine the place of origin of the heart beat;
3. Determine the direction of the blood flow.

DO NOT ALLOW THE EMBRYO AND YOLK TO DRY OUT. Apply several drops of warm HR.

The area on the yolk covered with blood vessels is called the area vasculosa. At this stage of development the blood in the vessels pick up oxygen and food for the embryo and dispose of embryonic waste. As the embryo grows this area increases. Refer to a diagram of the 48-hour chick.

Use your light to keep the embryo warm, and continue to keep it moist with the HR. Identify other prominent structures. Is the heart rate slowing down? Why?

C. Explantation of the chick heart rudiments and organ culture.

Obtain a 35 mm culture dish with sterile culture medium. In most tissue culture work the scientist is greatly concerned about maintaining sterile conditions to eliminate contamination of his cultures. The only part of this lab that need be sterile is the culture medium for the excised heart.

Prepare to remove the embryo including the area vasculosa to a sterile petri dish containing warm HR. (Why must the HR be kept warm?) Carefully cut around the outside of the area vasculosa, gently supporting the embryo with a pair of forceps, or hold the embryo with a forceps placed across the body below the heart. DO NOT LET THE EMBRYO DROP DOWN INTO THE YOLK. When the circular incision is complete remove the em-

bryo and area vasculosa to the sterile HR in the petri dish. Try to carry over as little yolk as possible. Pull the embryo through the solution to free it of excess yolk. Turn the embryo dorsal side down, yolk side up. The heart should then be uppermost.

An alternative method for removing the embryo is to cut out a Life-Saver shaped disk from filter paper and lay this disk on the area vasculosa. The hole in the middle should be approximately the size of the embryo and the entire disk should be slightly smaller than the area vasculosa. Make sure the filter paper is wet. Hold on to an edge of the paper and cut along the outside. The embryo can be moved to a petri dish by transferring the entire filter paper disk.

Dissect out the heart from above (anterior to) the truncus arteriosus (the main trunk vessel leaving the ventricle) and below (posterior to) the sinus venosus. This is best performed with glass needles and/or fine scissors and forceps. The easiest way to do this is to first sever the head portion of the embryo from the heart region. Then sever the posterior portion of the whole embryo from the heart region. Remove the heart region from the rest of the embryo. Usually you will remove the heart and digestive tract. Then the heart can finally be freed from all other tissue. Place the heart (with a pipet or forceps) into the 35 mm culture dish and label it "W."

Repeat this procedure, excising the whole heart, and then cut it into three regions. The anterior can be distinguished from the posterior if the heart is beating. If it does not beat at first, allow time for it to warm up. The beat in the whole heart begins at the sinus—thus the posterior end of the heart can be determined. Try to cut the heart into the three regions corresponding to the sinus, atrium, and the ventricle. Place the three regions of the heart into a 35 mm culture dish. (It is best to mark off three pie-shaped regions on the bottom of the dish and label A, V, S, *before* you put in pieces of heart.)

After careful observation (see below) cover and label your dish as to name, date, and time. Place the culture dish in a moist environment (plastic shoe box with wet cotton). Place the box in the incubator at 37.5°C.

If the media and explants are warmed sufficiently by the light, and if you have not injured the heart tissue, the whole heart and heart fragments will begin to beat. Observe. Record the rate of beating. Do all the fragments beat at the same rate? Do they beat at the same rate as the whole heart *in vivo? In vitro?*

If your techniques have been good, it is quite possible to keep the heart alive and beating for hours, days, and if fed, weeks. Make *at least one* observation after 18 hours of culture.

Using your experimental evidence from the organ culture of the heart, answer the following questions:

1. Is the heart beat an intrinsic property of the cardiac muscle, or is it a result of nervous or hormonal signals?
2. Does each section of the heart tube still have its own separate rate?
3. Are the new heart-beat rates that appear during development the result of all the heart cells changing their rates, or is the new rate merely imposed by the new section of the heart that is being incorporated?
4. Is the further morphogenesis of the heart programmed into the tissue itself, or does the heart develop its form in conjunction with other structures of the body and the fluid dynamics of the functioning circulatory system?

D. Suggestions for additional exercises.

During the 1981 meeting of ABLE at Stony Brook the participants in this workshop had the following suggestions for additional exercises. Unfortunately, I cannot give individuals names, but I thank everyone who participated and shared information so willingly.

1. To see if the rate of one piece of heart will influence the rate of another section of the heart, carefully align the pieces and keep them in contact while observing through the dissecting scope.
2. Now that it has been established that the heart does beat without the influence of nerves or hormones, try to add substances such as acetylcholine and epinephrine to the culture medium and look for heart-rate changes.

Instructors' Materials

Supplies and Materials

1. For each lab (20 students/lab):
   Whole-mount slides of chick—48-hour, and other stages.
   Chick embryos embedded in plastic may be used. I have my students submerge them in water for viewing, this eliminates effects of scratches in the plastic.
   Water bath set at 40–45°C for Howard's Ringers.
   2000 ml of Howard's Ringers in plastic squeeze bottles in water bath. (Keep an additional liter of Howard's Ringers available to fill squeeze bottles.)
   Plastic shoe box filled with moist cotton for culture dishes to be incubated.

Incubator set at 37.5°C with open dish of water to keep humidity as high as possible. Table-top models such as Carolina #70–1214 Egg Incubator will hold 100 eggs and can be used for culture dishes also. I use a Freas Low Temperature Incubator (GCA/Precision Scientific Model 816) for eggs and Table Top Model for culture dishes.

For each pair of students:

1 clean fingerbowl 4½ inch diameter (Carolina #74–1004 culture dish)
1 sterile 100 mm petri dish
2 sterile 35 mm culture dishes each with 2 ml of culture medium
1 wax pencil
1 pair watchmaker's forceps
1 pair of regular forceps
1 pair of fine scissors
Coplin jar filled with 70% alcohol and cotton on the bottom. If you are going to keep for another laboratory period, put cover on after lab. CAUTION STUDENTS—NO FLAMES NEAR ALCOHOL
3–4 Pyrex glass rods with tip drawn out into a 1–2 cm fine point. To create these glass dissecting tools, heat glass rods over Bunsen burner with wing tip. Rotate while heating, then pull out and break in the middle. Do not fire polish ends; you want sharp tips.
2–3 eggs 48-hours of incubation with mark indicating top during last rotation.

2. Solutions.

Howard's Ringer Solution, 2000 ml/lab

NaCl	14.40 g
$CaCl \cdot 2H_2O$	0.46 g
KCl	0.74 g
Distilled H_2O	to 2000 ml

Culture Medium (sterile), 1 bottle/lab

Horse Serum	5 ml
Medium 199	20 ml
Earle's Balanced Salt Solution	75 ml
Pen-Strep Concentrate	1 ml
	101 ml

All of the above can be purchased from GIBCO Laboratories, 3175 Staley Road, Grand Island, NY 14072. Hints: Buy Earle's Balanced Salt Solution (EBSS) (Cat. No. 310–4010) in 100 ml bottles. Then you can just pour off approximately 25 ml (into another sterile container) and add your other solutions directly to the EBSS using sterile techniques. EBSS has

a shelf life of one year at room temperature, but the Medium 199 (Cat. No. 320–1153) must be kept refrigerated, and has a storage life of two months. Upon receipt of the Horse Serum (Cat. No. 200–5060) I transfer 5 ml aliquots into sterile containers and freeze. The shelf life is six months. Pen-Strep is an antibiotic-antimycotic solution (100x) (Cat. No. 600520) which must be kept frozen and has a shelf life of six months. DO NOT MIX AND THEN STERILIZE. Instructors should use a sterile pipet to deliver 2 ml of the culture medium to the culture dishes. EBSS and Medium 199 are red because they contain Phenol Red and sodium bicarbonate. Complete listings of the contents of EBSS and Medium 199 are found in the GIBCO Laboratories Technical Brochure.

3. Eggs.

Fertile eggs purchased from a major biological supplier can cost $8.00 a dozen. Try your phone book for names of egg dealers and egg hatcheries. Some egg farmers keep a fertile hen house. Ask at health food stores or grocery co-ops for fertile eggs also. Eggs may be kept cool (not below 12°C) for about a week until time for incubation. Always keep the humidity high by placing pans of water in the refrigeration unit.

The amount of incubation time for a 55-hour embryo depends upon the pre-incubation treatment. If the eggs have not been refrigerated, then 55 hours of incubation would suffice. However, if the eggs have been refrigerated add 24 hours of incubation time to the desired hours of incubation. I usually figure three days incubation for 55 hour embryos after previous refrigeration. Incubation times will vary, but do not give up. I've had students remove the hearts of 35-hour embryos.

Again, during incubation maintain high humidity. I usually turn the eggs twice a day, although it is not strictly necessary. The morning before the lab, I place the eggs on their side and mark the top side with a straight line. Thus when the students crack their eggs as if they were going to cook a sunny side up egg, the embryo is at the top.

If during the laboratory a student breaks the yolk upon opening the egg, tell him to persist in finding the area vasculosa and, with luck, the chick. Because the yolk is so opaque it is difficult to find an embryo which is covered by yolk. On occasion by searching through the fingerbowl the lost embryo can be recovered, but usually the student fishes out the chalazae.

During the lab it is prudent to set up a "Liquid Egg Waste" container that can be emptied down the toilet. Egg shells can be disposed of in the normal trash.

Hint: For viewing the removed embryo use a black background on the dissecting scopes, and place the pod of the dissecting scope so that the control knobs are away from you, leaving more room for dissection.

4. Audio-visual aides.

I use the Carolina Bioreview Sheets on Chick Development as illustrations of structures to be located. The movie "Development of the Cardio-Vascular System of the Chick: The Heart" covers heart development from primitive streak stage. The terminology is too complex for an introductory course, but it does show an explanted heart and the rate changes when the heart is cut into pieces. If you are unsure of the fertility of the available eggs I recommend having the film available, because the students could answer the development questions posed in the laboratory exercise. The film is in color, 20 minutes long, and can be rented from Pennsylvania State University Audio-Visual Services.

References

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